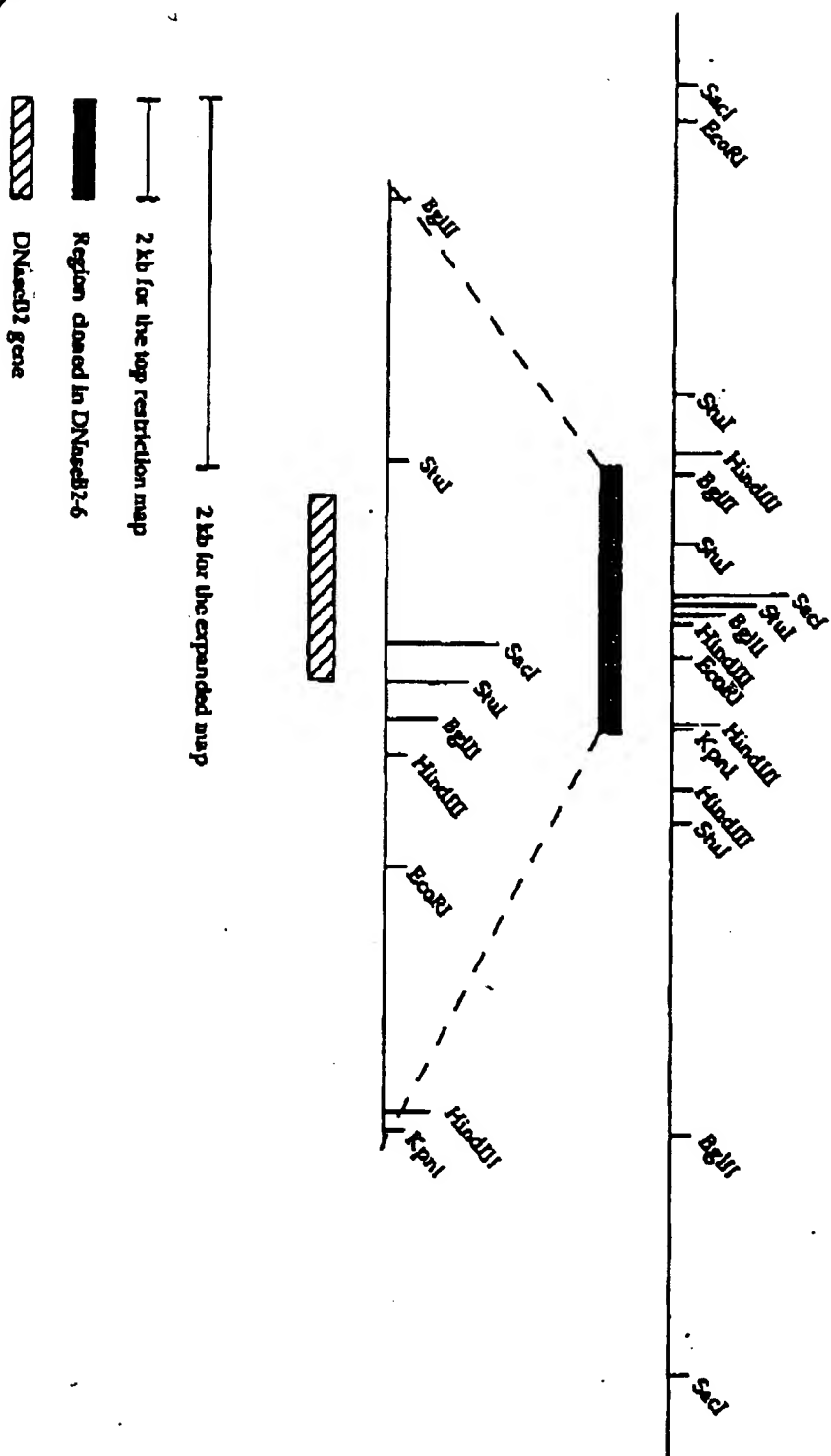


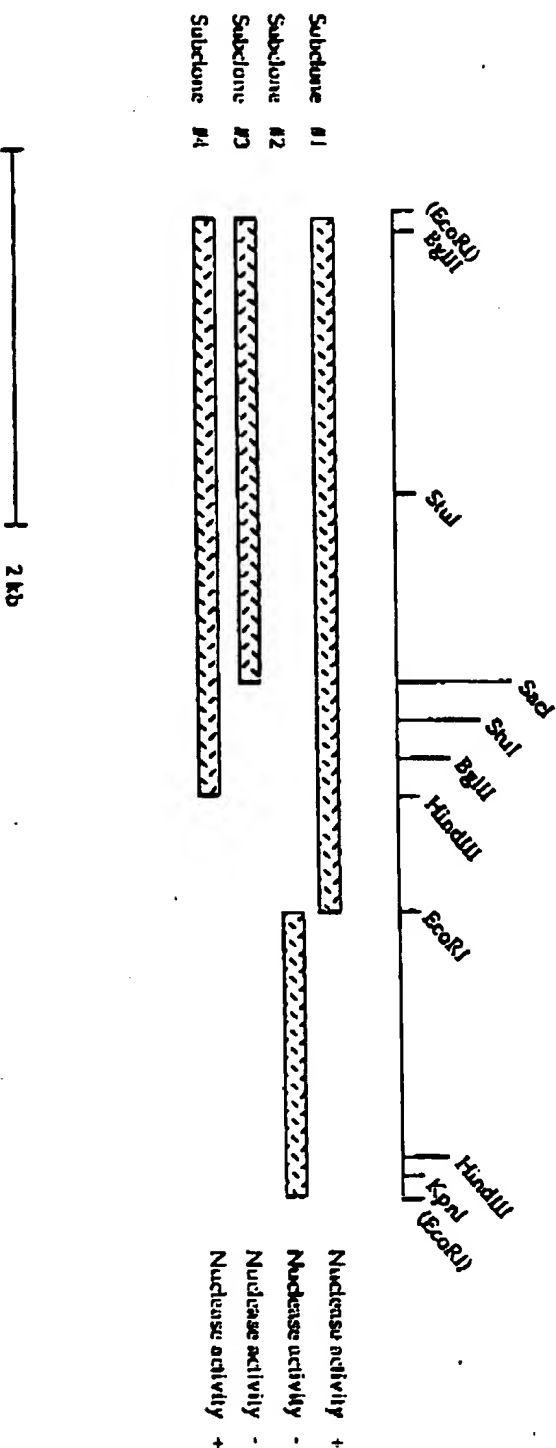
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Partial Restriction Map of DNaseB2 and its adjacent region  
in *Streptococcus pyogenes*



F16.1

## Subclones of DNaseB2 and its ability to produce active nuclease.



11/G. 2

(Linear) MAP of: Dnaseb2-6-3.Seq check: 2966 from: 1 to: 1083

REVERSE-COMPLEMENT of: Dnaseb2-6-No.Seq check: 8970 from: 1 to: 326  
DNASE B 2-6 CLONE SEQUENCE

With 149 enzymes: \*

MinOpen: 200

October 20, 1992 14:23 ..

(SEQ ID NO: 8, 9)

```

      T
      M S      MD      SM M      AX
      s p      sr      ps s      cc
      e E      ee      ee e      ce
      j j      j j      j j      j j
GACAACGGCTTCTTTTCTCCTTACTATCTCCTTAATTTTCATATTTTAAAAAACTATTGATAAAGTAACTAAGCTTATCTATGCTTACT
1-----100
CTGTTCCGGAAGAAAAAGACCAATGATACAGGAAATTAAGTATAAAAAATTTTGTATAACTATTGATCAATTCATTCCCATATGATACCAATCA

```

a:  
b:  
c:

```

      T
      t h s
      n i 1 u d A
      d n 1 3 p l
      e f 1 A n w
      j j 1 j j j
TACGAAATTAGAAAAAGAGGACAAGCATATGAATCTACTTGCATCAAGACGGTTTTTTCTAAAAATGTCGGCTAGTAAAAATTTCAATGCTAGCTCTT
101-----200
ATCGCTTTAATCTTTTCTCCTGTTCTATACTTAGATGAACCTAGTCTGCCCAAAAAAGATTTTACAGCCGATCATTTTAAAAAGTTACCATCGAGAA

```

a:  
b:  
c:

MetAsnLeuGlySerArgArgValPheSerLysLysCysArgLeuValLysPheSerMetValAlaLeu

```

      T
      t
      C B      M T      EUN      M
      y B v e      e p      cb1      i
      i g i i      i 4      ca1      n n
      j l j j      i 5      321      p h
      j i i i      i j      161      i a
      j j j j      j j      j j j      j j
GTATCAGCCACAATGGCTGTAAACAACATCACACTTGAAATACTGCACCTGGCAGCAAAACACAGGCTCAAATGATGTTGTTCTAAATGATGGCCAA
201-----300
CATAGTCGGTGTACCGACATTGTTGTCAGTGTGAACCTTTATGACGTGACCGTCTGTTGTGTCCAGAGTTACTACAACAAGATTTACTACCGCGTT

```

a:  
b:  
c:

ValSerAlaThrMetAlaValThrThrValThrLeuGluAsnThrAlaLeuAlaArgGlnThrGlnValSerAsnAspValValLeuAsnAspGlyAlaSer

```

      T
      t h
      C B      C      C      E
      vR 1      Av      GVRMS      c
      is 1      li      sisp      o n
      Qa 1      wj      uase      n
      j j      j j      j j j      j
GCAAGTACCTAAACGAAGCATTAGCTTGACATTCAATGACAGTCTTAACCTATTACAAAACCTTACGTTACTAGTCAGATTACTCCAGCACTCTTCTCTAA
301-----400
CGTTCAAGGATTGCTTCGTAATCGAACCTGTAAGTTACTGTGAGGATTGATAATGTTTGAATCCATGATCAGTCTAATGAGGTCTGAGAAAGGATT

```

a:  
b:  
c:

LysTyrLeuAsnGluAlaLeuAlaTrpThrPheAsnAspSerProAsnTyrTyrLysThrLeuGlyThrSerGlnIleThrProAlaLeuPheProLys

F16.3

SP	ML	Y	HP
SO	1	SP	2
rk	1	E	6
11	1		

S	U	B	M	A	M	C	M
f	b	c	P	f	a	a	a
a	a	d	l	l	l	l	l
n	2	e	e	l	l	l	l
1	1	x	l	l	l	l	l

				S
M T	F	NN		P
a s	n	i l N	C81N	
e p	U	naKtS	Ava2gS	
I 4	4	PIhhp	lIn8!a	
I 5	N	llkth	wJ!6ac	
I f	J	IIIII	IIIIII	
		/ / //	/ ///	

a:  
b:  
c: ArgAspGlnValGlyGlyMetArgTyrThrGluGlnArgAlaGlnGluTyrPheGluValAsnArgAspGlyTyrLeuTyrTyrGluValAlaProIle

N C H C  
 f i b  
 n l i  
 f u j  
 i i i

TACAACCCAGACAGGTTGATTCCAAGAGCTGTCGGTATCAATGCAATCTTCTGATAATACCATCAACGAGAAAGTATTAGTTTACAACACAGCTAATG  
 801 ----- 900  
 ATGTTGCTCTGCTCAACTAAGGTTCTCCACAGCACCATTAGTTACGTTAGAAGACTATTATGGTAGTCTCTTTCATAATCAAATGTTGTGTCGATTAC

a:  
 b:  
 c: TyrAsnAlaAspGluLeuIleProArgAlaValValValSerMetGlnSerSerAspAsnThrIleAsnGluLysValLeuValTyrAsnThrAlaAsnGly -

C H C C C  
 v i v i v i v i v i  
 i s s s s s  
 j e q e e e  
 i i i i i i

6CTACACCTAACTACCATAACGGTACACCTACTCAAAAATAATACCAAAAGGCTAGACCTCTGCTCACTAGGCTAGCTTTTACATCAAAAAAGCA  
 901 ----- 1000  
 CGATGTGTAATGTATGCTATGCCATGTGGATGAGTTTATTATGCTTTCCGATCTCGACACGAGTGATCCGATCGAAAAATGATGTTTTTTCGT

a:  
 b:  
 c: TyrThrIleAsnTyrHisAsnGlyThrProThrGlnLysEnd

M C T  
 e e s  
 e f p  
 e i e  
 i x i

ATGACTATAGAAAGTAAAAAATACTAGAAAAAACAATGATTGCCGTATTGCTTTTATGAATTTGTGCAAAAACCAAAAAAGC  
 1001 ----- 1083  
 TACTGATATCTTTTCATTTTATGATCTTTTTCGTTACTAAGCCAGTAACCAAAAAATACTTAAACACGTTTTTCGTTTTTTCG

a:  
 b:  
 c: MetAsnLeuCysLysLysGlnLysSer -  
 MetIleAlaValIleAlaPheTyrGluPheValGlnLysAlaLysLys777 -

## Enzymes that do cut:

AacI	AflIII	AluI	AluI	BamI	BclI	BglI	Bsp1286I	BsrI	CviI	CviII	DdeI	DpnI
DraI	Eco31I	EcoRI	Fnu4HI	FokI	GsuI	HaeI	HaeIII	HpaI	HgiAI	HhaI	HinfI	HirPI
HaeI	HaeIII	HaeIII	HboI	HluI	HmaI	HniI	HseI	HdeI	HleI	HspHI	PleI	RsaI
SacI	Sau3AI	SfaNI	SpeI	SphI	StuI	ThaI	Tsp45I	TspEI	Tth111I	Uba26I	XbaI	

## Enzymes that do not cut:

AatII	AflI	AhaI	AluNI	AocI	ApaI	ApalI	Asp700I	Asp718I	AsuI	AvaI	AvaII	AvrII
BclI	BamHI	BanI	BbeI	BbvI	BbvII	BclI	BglII	BsmI	BspMI	BspMI	BspMI	BsrI
BstEII	BstNI	BstXI	CfrI	Cfr10I	Clal	DraI	DraIII	DsaI	Eco47III	Eco57I	Eco78I	EcoRI
EcoRI	EcoRV	EspI	FinI	FinI	PapI	GdlI	HaeII	HgiEII	HincII	HindIII	HpaI	HpaII
HphI	KpnI	Ksp32I	HfeI	HaeI	HaeI	HclI	HcoI	HheI	HlaIV	HolI	HruI	HstI
Hsp6II	PfIM	PmeCI	PpuMI	PssI	PstI	PvuI	PvuII	RsrI	SacII	SatI	Sau96I	ScaI
SciI	ScrPI	SecI	SfiI	SmaI	SmaII	SplI	SsoI	SspI	StyI	TaqI	TaqII	TaqII
Tth111I	VspI	XbaI	XhoI	XhoII	XmaI	XmaIII						

FIG. 3, cont

## FIGURE 4

AMINO ACID SEQUENCE OF CLONED S. pyogenes DNase

R-Q-T-Q-V-S-N-D-V-V-L-N-D-G-A-S-K-Y-L-N-  
E-A-L-A-W-T-F-N-D-S-P-N-Y-Y-K-T-L-G-T-S-  
Q-I-T-P-A-L-F-P-K-A-G-D-I-L-Y-S-K-L-D-E-  
L-G-R-T-R-T-A-R-G-T-L-T-Y-A-N-V-E-G-S-Y-  
G-V-R-Q-S-F-G-K-N-Q-N-P-A-G-W-T-G-N-P-N-  
H-V-K-Y-K-I-E-W-L-N-G-L-S-Y-V-G-D-F-W-N-  
R-S-H-L-I-A-D-S-L-G-G-D-A-L-R-V-N-A-V-T-  
G-T-R-T-Q-N-V-G-G-R-D-Q-K-G-G-M-R-Y-T-E-  
Q-R-A-Q-E-W-L-E-A-N-R-D-G-Y-L-Y-Y-E-V-A-  
P-I-Y-N-A-D-E-L-I-P-R-A-V-V-V-S-M-Q-S-S-  
D-N-T-I-N-E-K-V-L-V-Y-N-T-A-N-G-Y-T-I-N-  
Y-H-N-G-T-P-T-Q-K

(SEQ ID NO: 9)

## PCR OLIGONUCLEOTIDE

5' TAACGGATCCGAATCTACTTGGATCAAGACGGGTTTTTCT 3' (SEQ ID No. 2)

```

1  ATGGATCCGAATCTACTTGGATCAAGACGGGTTTTTCTAAAAATGTCGGCTAGTAAAA
   .....+.....+.....+.....+.....+.....+.....+.....+.....+ 60
   TACCTAGGCTTAGATGAACCTAGTTCTGCCCAAAAAGATTTTTTACAGCGGATCATTTT

MetAspProAsnLeuLeuGlySerArgArgValPheSerLysLysCysArgLeuValLys -
61  TTTTCAATGGTAGCTCTTGTATCAGCCACAATGGCTGTAACAACAGTCACACTTGAAAAAT
   .....+.....+.....+.....+.....+.....+.....+.....+.....+ 120
   AAAAGTTACGATCGAGAACATAGTCGGTGTTACCGACATTGTTGTCAGTGTGAACTTTA

PheSerMetValAlaLeuValSerAlaThrMetAlaValThrThrValThrLeuGluAsn -
121  ACTGCACTGGCAGCACAACACAGGTCTCAAATGATGTTGTTCTAAATGATGGCGCAAGC
   .....+.....+.....+.....+.....+.....+.....+.....+.....+ 180
   TGACGTGACCGTGCTGTTTGTCTCCAGAGTTTACTACAACAAGATTTACTACCGCGTTCG

ThrAlaLeuAlaArgGlnThrGlnValSerAsnAspValValLeuAsnAspGlyAlaSer -
181  AAGTACCTAAACGAAGCATTAGCTTGGACATTCAATGACAGTCCTAACTATTACAAAAC
   .....+.....+.....+.....+.....+.....+.....+.....+.....+ 240
   TTCATGGATTTCGTTTCGTAATCGAACCTGTAAGTTACTGTCAGGATTGATAATGTTTTGA

LysTyrLeuAsnGluAlaLeuAlaTrpThrPheAsnAspSerProAsnTyrTyrLysThr -
241  TTAGTACTAGTCAGATTACTCCAGCACTCTTTCCTAAAGCAGGAGATATTCTCTATAGC
   .....+.....+.....+.....+.....+.....+.....+.....+.....+ 300
   AATCCATGATCAGTCTAATGAGGTCGTGAGAAAGGATTTTCCTCTATAAGAGATATCG

LeuGlyThrSerGlnIleThrProAlaLeuPheProLysAlaGlyAspIleLeuTyrSer -
301  AAATTAGATGAGTTAGGAAGGACGGTACTGCTAGAGGTACATTGACTTATGCCAATGTT
   .....+.....+.....+.....+.....+.....+.....+.....+.....+ 360
   TTAAATCTACTCAATCCTTCCTGGCATGACGATCTCCATGTAAGTGAATACGGTTACAA

LysLeuAspGluLeuGlyArgThrArgThrAlaArgGlyThrLeuThrTyrAlaAsnVal -
361  GAAGGTAGCTACGGTGTTAGACAATCTTTCCGTAAAAATCAAAACCCCGCAGGATGGACT
   .....+.....+.....+.....+.....+.....+.....+.....+.....+ 420
   CTTCCATCGATGCCACAATCTGTTAGAAAGCCATTTTACTTTTGGGGCGTCTACCTGA

GluGlySerTyrGlyValArgGlnSerPheGlyLysAsnGlnAsnProAlaGlyTrpThr -
421  GGAACCCCTAATCATGTCAAATATAAAATTGAATGGTTAAATGGTCTATCTTATGTCGGA
   .....+.....+.....+.....+.....+.....+.....+.....+.....+ 480
   CCTTTGGGATTAGTACAGTTTATATTTTAACTTACCAATTTACCAGATAGAATACAGCCT

GlyAsnProAsnHisValLysTyrLysIleGluTrpLeuAsnGlyLeuSerTyrValGly -

```

FIG. 5

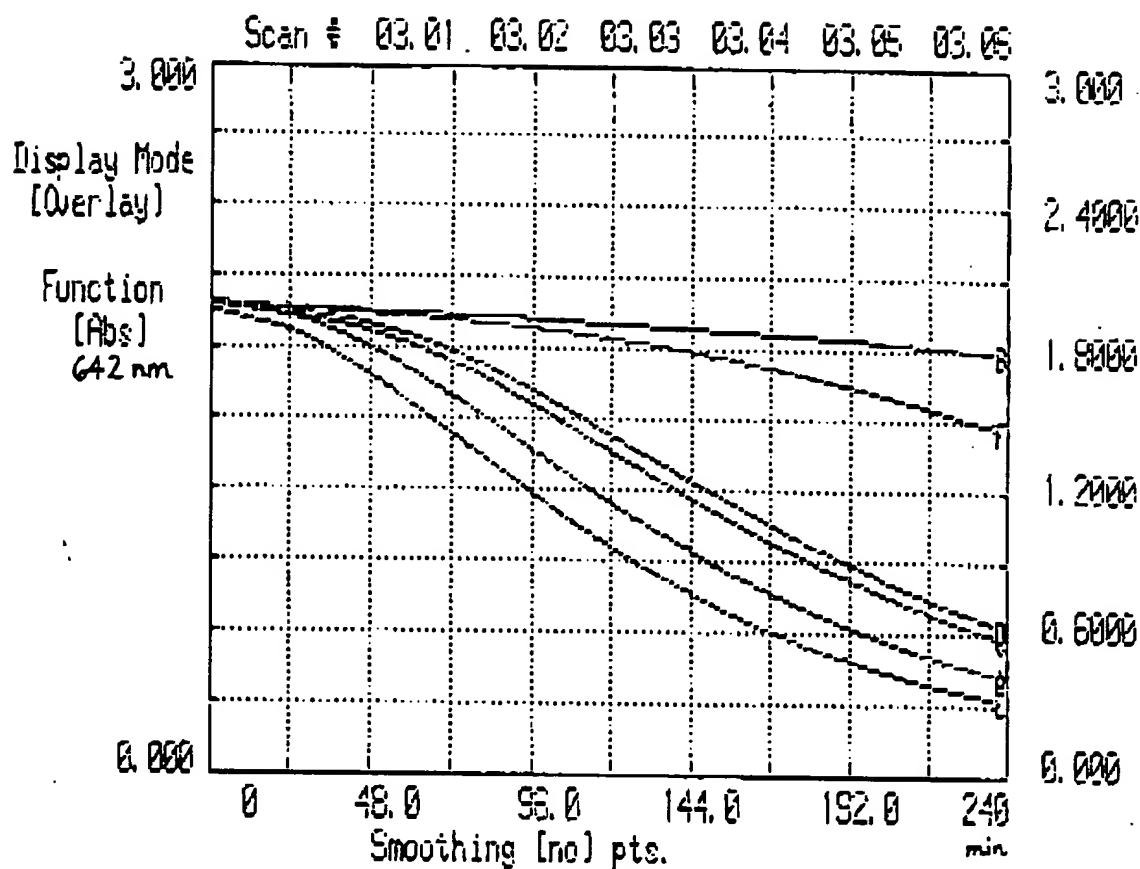
481 GATTTCCTGGAATAGAAGTCATCTCATTGCAGATAGTCTCGGTGGAGATGCCACTCAGAGTC 340  
 CTAAAGACCTTATCTTCAGTAGAGTAACGTCTATCAGAGCCACCTCTACGTGAGTCTCAG  
 AspPheTrpAsnArgSerHisLeuIleAlaAspSerLeuGlyGlyAspAlaLeuArgVal -  
 541 AATGCCGTTACAGGAACACGTACCCAAATGTAGGAGGTCGTGACCAAAAAGCGGCATC 600  
 TTACGGCAATGTCCTTGTGCATGGGTTTACATCCTCCAGCACTGGTTTTTCCGCCGTAC  
 AsnAlaValThrGlyThrArgThrGlnAsnValGlyGlyArgAspGlnLysGlyGlyMet -  
 601 CGCTATACCGAACAAAGAGCTCAAGAATCGTTAGAAGCAAATCGTGATGGCTATCTTTAT 660  
 GCGATATGGCTTGTTCTCGAGTTCTTACCAATCTTCGTTTAGCACTACCGATAGAAATA  
 ArgTyrThrGluGlnArgAlaGlnGluTrpLeuGluAlaAsnArgAspGlyTyrLeuTyr -  
 661 TATGAAGTCGCTCCAATCTACAACGCAGACGAGTTGATTCCAAGAGCTGTCGTGCTATCA 720  
 ATACTTCAGCGAGGTTAGATGTTGCGTCTGCTCAACTAAGGTTCTCGACAGCACCATAGT  
 TyrGluValAlaProIleTyrAsnAlaAspGluLeuIleProArgAlaValValValSer -  
 721 ATGCAATCTTCTGATAATACCATCAACGAGAAAGTATTAGTTTACAACACAGCTAATGCC 780  
 TACGTTAGAAGACTATTATGGTAGTTGCTCTTTCATAATCAAATGTTGTCTCCATLACCG  
 MetGlnSerSerAspAsnThrIleAsnGluLysValLeuValTyrAsnThrAlaAsnGly -  
 781 TACACCATTAACTACGATAACGGGTACACCTACTCAAAAATAATACCAAAGGCTAGACCT 840  
 ATGTGGTAATTGATGGTATTGCCATGTGGATGAGTTTTTATTATGGTTTTCCGATCTGGA  
 TyrThrIleAsnTyrHisAsnGlyThrProThrGlnLysEndTyrGlnLysAlaArgPro -  
 841 CTGCTCACTAGCCCTAGCTTTTTACATCAAAAAAGCAATGACTATAGAAAGTAAAAATA 900  
 GACGAGTGATCCGATCGAAAAATGTAGTTTTTTTCGTTACTGATATCTTTCATTTTTAT  
 LeuLeuThrArgProSerPheLeuHisGlnLysLysGlnEnd -  
 901 CTAGAAAAAGCAATGATTGCCGTCATTGCCCCGGTCCGACCCGG 944 (SEQ ID No: 11)  
 GATCTTTTTCGTTACTAACGGCAGTAACGGGGCCAGCTGGGCC

3' TCTTTTTCGTTACTAACGGCAGTAACGGGGCCAGCTGGGCC 3' (SEQ ID No: 3)

PCR OLIGONUCLEOTIDE

FIG. 5 cont.





A = Streptonase B  
 B = Streptonase B + Antibody  
 C = DNase1-1

D = DNase1-1 + Antibody  
 E = DNase B2-6  
 F = DNase B2-6 + Antibody

FIG. 6

1 GACNAGCGCTTCTTTTCTGCTTACTATGCTGCTTAAATTTTCATATATTTTAAANAANAACCTAIIIGATAAACTAGTTAAGTAAAGCGTATACTATGCTTAGT -10  
-35  
101 TAGCGMAATTAGANAAGNGGACNAGCGCTATGTAATCTACTTGGATCAAGACGCGGTTTTTCTTAANAANAATGCTGCTAGTAANAATTTTCAATGCTAGCTCTT

(SEQ ID NO: 10)

Consensus sequence of Escherichia coli promoter region:

-35                      -10  
tcttgcacat              tataat

FIG: 7

Correlation Curve of Activity Assays Based  
On Recombinant and Natural (Streptolysin B)  
DNase B

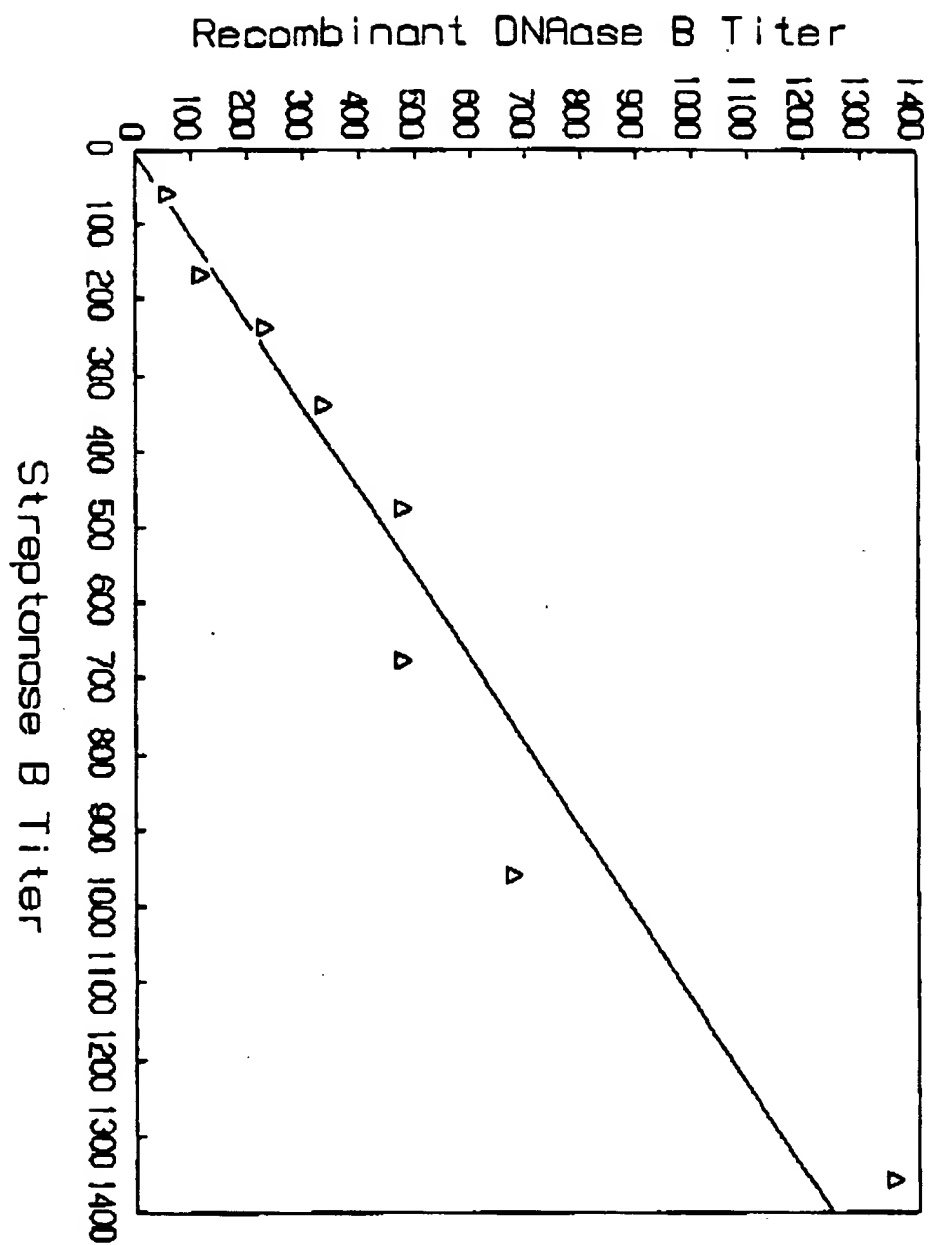


FIG. 8

# MITOGENIC ASSAY WITH MOUSE LYMPHOCYTES

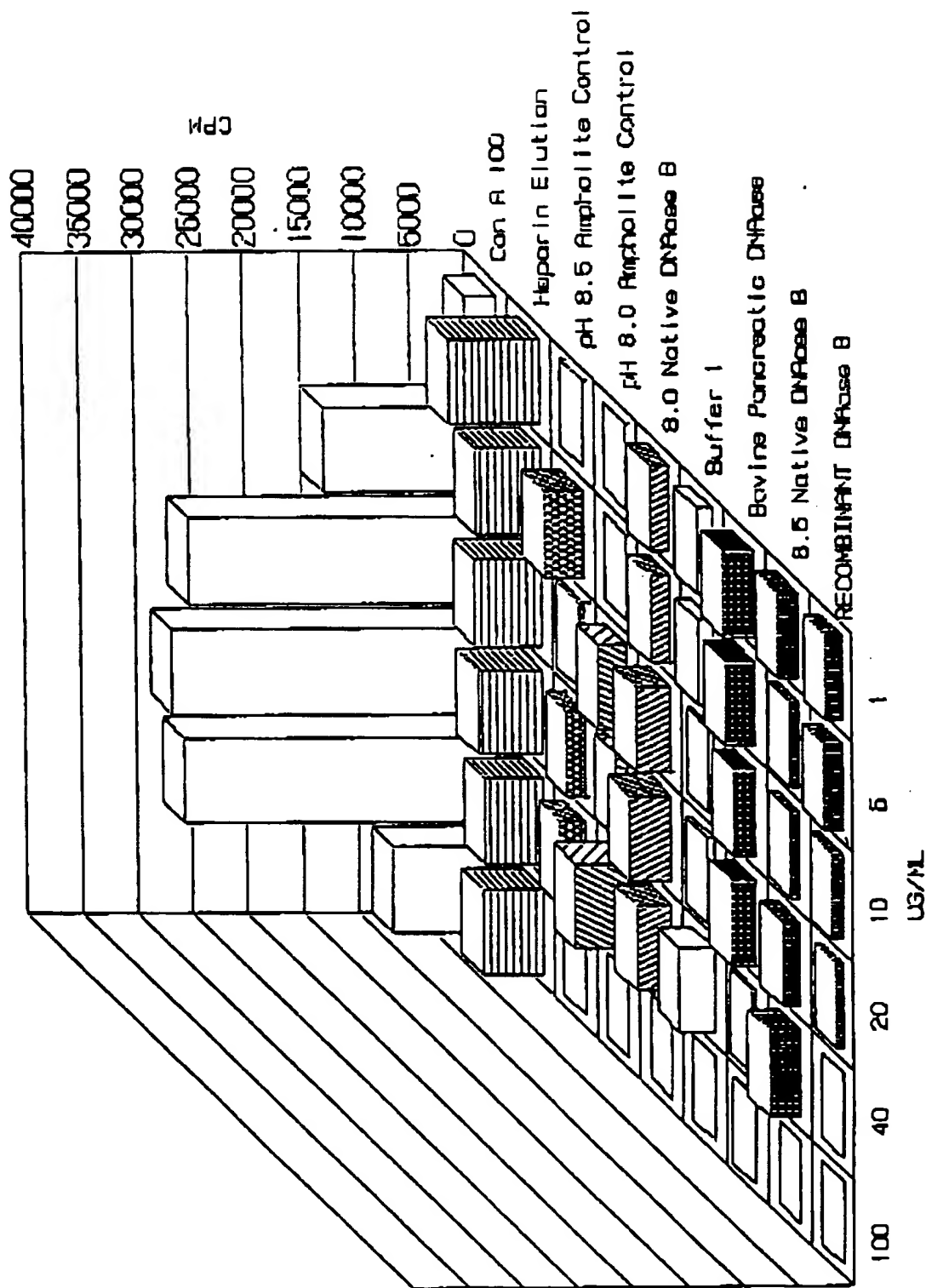


FIG 9

SEQUENCE OF CONSTRUCTION PRODUCING DNASE B  
PROCESSED IDENTICALLY TO NATURAL DNASE B

PCR OLIGONUCLEOTIDE (SEQ ID NO: 12)

5'AGGCAATGGATCCGAACCTGCTGGGTTCCCGTCGCTGTTTTCTCCAAAAATGCCGTCTGGTTAAATTCTCCAT  
 ATGGATCCGAACCTGCTGGGTTCCCGTCGCTGTTTTCTCCAAAAATGCCGTCTGGTTAAATTCTCCAT  
 1 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 60  
 TACCTAGGCTTAGATGAACCTAGTTCTGCCCAAAAAGATTTTTTACAGCCOATCATTTTAAAAGTTA  
 MetAspProAsnLeuLeuGlySerArgArgValPheSerLysLysCysArgLeuValLysPheSerMet -  
 GGTTCCTCTGCTTTCCGCTACCATGGCTGTTACCACCGTTACCGCTGGAAAACACCGCTCT  
 GGTTCCTCTGCTTTCCGCTACCATGGCTGTTACCACCGTTACCGCTGGAAAACACCGCTCT  
 61 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 120  
 CCATCGAAGCATAGTGGGTGTACCGACATTGTTGTCACTGTGAACCTTTATGACGTGA  
 ValAlaLeuValSerAlaThrMetAlaValThrThrValThrLeuGluAsnThrAlaLeu -  
 GGCT\*\*\*CAGACACAGGTCTCAAATGATGTTGTTCTAAATGATGGCGCAAGCAAGTACCT  
 GGCT\*\*\*CAGACACAGGTCTCAAATGATGTTGTTCTAAATGATGGCGCAAGCAAGTACCT  
 121 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 180  
 CCGTGCTGTTTGTGTCCAGAGTTTACTACAACAAGATTACTACCGCGTTCTGTTTCATGCA  
 AlaArgGlnThrGlnValSerAsnAspValValLeuAsnAspGlyAlaSerLysTyrLeu -  
 AAACGAAGCATTAGCTTGGACATTCAATGACAGTCTTAATATTACAAAACCTTTAGGTAC  
 181 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 240  
 TTGCTTCGTAATCGAACCTGTAAGTTACTGTCAGGATTGATAATGTTTTCAAATCCATG  
 AsnGluAlaLeuAlaTrpThrPheAsnAspSerProAsnTyrTyrLysThrLeuGlyThr -  
 TAGTCAGATTACTCCAGCACTGTTTCTAAAGCAGGACATATTCTCTATAGCAAATTAGA  
 241 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 300  
 ATCAGTCTAATGAGGTCGTGAGAAAGGATTTGCTCCTCTATAAGAGATATCGTTTAATCT  
 SerGlnIleThrProAlaLeuPheProLysAlaGlyAspIleLeuTyrSerLysLeuAsp -  
 TGAGTTAGGAAGACCGCTACTGCTAGAGGTACATTGACTTATGCCAATGTTGAAGGTAG  
 301 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 360  
 ACTCAATCCTTCCTGCGCATGCGATCTCCATGTAAGTGAATACGGTTACAACTTCCATC  
 GluLeuGlyArgThrArgThrAlaArgGlyThrLeuThrTyrAlaAsnValGluGlySer -  
 CTACCGTGTAGACAATCTTTCCGTAAAAATCAAAACCCCGCAGGATGGACTGGAACCC  
 361 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 420  
 GATGCCACAATCTGTTAGAAAGCCATTTTATGTTTTGGGGCGTCTACCTGACCTTTGGG  
 TyrGlyValArgGlnSerPheGlyLysAsnGlnAsnProAlaGlyTrpThrGlyAsnPro -  
 TAATCATGTCAAATATAAAATTGAATGTTAAATGGTCTATCTTATGTCGGAGATTTCTG  
 421 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 480  
 ATTAGTACAGTTTATATTTTAACTTACCAATTTACCAGATAGAATACAGCCTCTAAAGAC  
 AsnHisValLysTyrLysIleGluTrpLeuAsnGlyLeuSerTyrValGlyAspPheTrp -  
 GAATACAAGTCATCTCAATGCGATAGTCTCGGTGGAGATGCACTCAGAGTCAATGCCGT  
 481 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 540  
 CITATCTTCAGTAGAGTAACGTCTATCAGAGCCACCTCTACGTGAGTCTCAGTTACCGCA  
 AsnArgSerHisLeuIleAlaAspSerLeuGlyGlyAspAlaLeuArgValAsnAlaVal -

FIG. 10

F16. 10, cont.

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